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OBJECTIVE METHOD FOR DETERMINING THE
CONCENTRATION OF A BACTERIAL AEROSOL

V. S. Kiktenko, et al

Foreign Technology Division
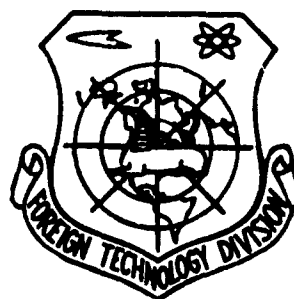Wright-Patterson Air Force Base, Ohio

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In order to determine the microorganism content in air, a
large number of devices based on various principles has been
proposed for catching bacteria. Most of the devices do not have
sufficient effectiveness with respect to determining the particles
of the bacterial and viral aerosols.

A comparative analysis of the effectiveness of the various
bacteria traps cannot be accomplished due to the absence of
objective methods for determining the bacterial aerosol concen-
trations. The most accurate data which describe the actual
effectiveness of the devices could have been obtained by comparing
the obtained results with the determination data on the bacterial
aerosol concentration per unit volume of air in the period
samples were taken. However, the existing methods for a qualita-
tive description of the aerosol, in addition to being tedious
and time consuming, to a considerable degree are based on subjec-
tive methods of estimation, which does not provide for accurate
results of studies.

We attempted to apply the objective method for determining the particle concentration in bacterial aerosols, based on using flow-through ultramicroscope VDK. Based on the latter we developed a highly sensitive device with an electromechanical counter, which permits us to count the number of aerosol particles passing through a cuvette automatically. Using the new method for determining the aerosol concentration, we carried out a comparative evaluation of the traps property of the devices designed by V. S. Kiktenko, S. S. Rechmenskiy, P. P. D'yakonov, A. Ye. Vershigora, and N. M. Rudenko, and of the soluble filters made from gelatin foam, and also cotton filters impregnated with a 3% solution of gelatin and vaseline mixture, i.e., devices based on the filtration of aerosols through the liquid and dense soluble filters, and also on the settling of aerosols by the dispersed liquid. The procedure for setting up and using the devices was in accordance with the instructions set up by the authors of these bacteria traps. In the experiments we produced droplet-type and dense aerosol phases of Chromobacterium prodigiosum (strain No. 1266) with particle dispersion from 0.6 to 3.4 μ and Bacillus subtilis (strain No. 8236) with the average particle radius value equal to 5 μ. Bacterial suspensions containing 0.5 billion microbe cells were prepared from a physiological solution with an addition of 15% glycerine and 5% sucrose solution; in preparing suspensions of chromobacterium prodigiosum bacillus we also used gelatin phosphate buffer (gelatin - 2 g, Na_2HPO_4 - 4 g, distilled water - 1000 m𝑙; pH - 7.0). Suspension containing Bacillus subtili spores was heated with shaking at 70-80° for a period of 5-10 min before use. The sampling was carried out simultaneously by 4 bacteria traps, 10 liters of air was passed through each, the sampling period lasted 1-3 min.

The study results are presented in Table 5.

Table 5. Comparative effectiveness evaluation of the bacteria traps (average data from 32 series of studies).

No.	Devices	Average number of micro-organisms determined in 1 l of air	
		Chromobacterium prodigiosum bacillus	Bacillus subtilis
	Bacteria-catcher designs:		
1	v. S. Kiktenko.....................	5,581	6,286
2	S. S. Richmenskiy.................	3,189	4,281
3	P. P. D'yakonov...................	3,081	3,862
4	N. M. Rudenko.....................	1,859	2,303
5	A. Ye. Vershigora.................	1,451	2,402
6	Soluble filters made from gelatin foam......................	152	1,922
7	Cotton filters:		
	a) dry cotton wool..............	21	861
	b) dry glass wool..............	42	893
	c) glass wool impregnated with a 3% solution of gelatin and vaseline mixture.............	37,218	42,680
	d) cotton wool impregnated with a 3% solution of gelatin and vaseline mixture.............	26,534	31,871

As seen from the presented data, the greatest number of microorganisms per 1 l of air was determined by means of the impregnated cotton filters. The insignificant number of microorganisms determined on the cotton wool and glass wool filters without impregnation is evidently explained by death of Chromobacterium prodigiosum bacillus, which is supported by the results of the study of Bacillus subtilis. We should also take into account the difficulty connected with washing out the microorganisms from the cotton.

Data presented in Table 5 obtained their confirmation during the study of the retaining property of devices, using the nephelometry method with respect to the oil mist particles and Chromobacterium prodigiosum bacillus aerosol. The particle size of the oil mist was 0.31-0.34 μ.

The study results are presented in Table 6.

Table 6. The slip-through factor in various devices with respect to oil mist and bacterial aerosol particles.

Filter material	Amount of material (in g/ml)	Slip-through factor (in %)	
		Oil mist	Bacterial aerosol
Glass wool......................	10	2.1	5.5
Cotton wool.....................	5	0.08	1.05
Glass wool impregnated with a 3% solution of gelatin and vaseline mixture................	10 + 12 ml of impregnation	8.1	0.35
Impregnated cotton wool.........	5 + 12 ml of impregnation	0.1	0.02₅
Bacteria traps of different designs........................	4-40 ml of filtered liquid	42-48	

As shown by the data (presented in Table 6) the highest slip-through factor was observed in the devices of various designs; the lowest retaining property was noted in the soluble filters made from gelatin foam in which the slip-through factor comprised 92-98%. The device designed by V. S. Kiktenko had a slip-through factor in the range of 42-48% with respect to the oil mist.

Bacterial aerosol concentration in the chamber was determined during the sampling period by means of a photoelectronic device (Table 7).

Table 7. Aerosol particle concentration in the chamber during the air sampling period (average data based on 18 series of studies).

Readings by the photoelectronic particle counter (in 1 *l* of air)

Immediately after spraying	after 3 min	5 min	10 min	15 min	20 min	25 min	30 min
1 441 700	1 438 286	1 396 018	1 106 321	1 028 046	987654	967304	804206

The aerosol concentration was calcu. ted by the formula:

$$n = \frac{N}{V} = \frac{d \cdot N}{W},$$

where N – number of counted flashes (particles); W – aerosol volume; d – constant of the given diaphragm opening with a given optical system; V – aerosol volume in which the number of particles was counted.

As seen from the data presented in Table 7 the aerosol concentration in the chamber dropped only 5% ten minutes after the suspension was sprayed relative to its initial concentration determined immediately after the suspension was sprayed. Consequently, no significant change in aerosol concentration was noted during the sampling period.

Considering the fact that the photoelectronic counter determines the total concentration of aerosol whose particles do not necessarily contain the microorganisms, it seemed advisable to compute the approximate number of particles containing the microbe cells per unit volume.

To determine the total number of particles of the bacterial aerosol containing microbe cells, we used the method of drying the droplet phase of the bacterial aerosol by passing it through

the silica gel desiccating columns. Concentration of the bacterial
aerosol particles (solid or droplet-nuclear fraction) determined
by means of the photoelectronic particle counter comprised on
the average 43,250/1 l of air in the chamber during the sampling
by the devices, which comprised about 3% of the total number of
aerosol particles. Using this method to determine the number
of aerosol particles containing the microorganisms and, also,
taking into account the evaluation results of the device effec-
tiveness, it is possible to determine an approximate quantity
which characterizes the trapping property of a particular device.
Thus, taking into consideration the data in Table 5, we find that
with the aid of the bacteria trap with cotton filters impregnated
with a 3% solution of gelatin and vaseline mixture, 89% of all
microorganisms found in a unit volume of the chamber air are
determined; the trappingproperty of other devices varies in the
range of 0.3 to 14%. It is necessary to indicate that the
presented data do not include the percent of microorganisms that
die during the spraying and sampling using the bacteria traps
of various designs.

CONCLUSIONS

1. The methods used by various authors to determine the
effectiveness of the bacteria traps based on the sedimentation
and filtration principle rely on subjective methods of estimation,
which affects the accuracy of the obtained results.

2. We propose an objective method for estimating the
effectiveness of the bacteria traps, using the photoelectronic
particle counter which enables one to determine the traps property
of devices taking into account the concentration of the bacterial
aerosol particles during sampling.